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(57) Abstract

A phosphopeptide or a salt thereof the phosphopeptide having from 5 to 30 amino acids including the sequence A-B-C-D-E where A, B, C, D and E are independently phosphoserine, phosphothreonine, phosphotyrosine, phosphohistidine, glutamate and aspartate and compositions particularly compositions to teeth including same

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1 TITLE: PHOSPHOPEPTIDES

2 This invention relates to phosphopeptides and
3 compositions containing same.

4 This invention also relates to caries and gingivitis
5 inhibition.

6 The present invention provides a phosphopeptide or a
7 salt thereof, the phosphopeptide having from 5 to 30 amino
8 acids including the sequence

9 A-B-C-D-E

10 where A,B,C,D and E are independently phosphoserine,
11 phosphothreonine, phosphotyrosine, phosphohistidine,
12 glutamate and aspartate.

13 Preferred phosphopeptides are those wherein A,B and C
14 are independently phosphoserine, phosphothreonine,
15 phosphotyrosine and phosphohistidine and D and E are
16 independently phosphoserine, phosphothreonine, glutamate and
17 aspartate.

18 Particularly preferred phosphopeptides are those where
19 A,B and C are phosphoserine and D and E are glutamate.

20 The phosphopeptide is preferably in substantially pure
21 form.

22 The phosphopeptides of the present invention or their
23 salts may have utility in the treatment or inhibition of (i)
24 dental diseases such as caries, gingivitis and periodontal
25 disease, (ii) rarefying bone diseases such as osteoporosis
26 and osteomalacia and (iii) diseases relating to
27 malabsorption of minerals.

28 Accordingly, the present invention provides a
29 composition comprising a peptide or a salt thereof in
30 accordance with this invention and a physiologically
31 acceptable diluent.

32 The composition may be in the form of a pharmaceutical
33 composition.

34 The composition may be orally ingestible.

35 A mixture of phosphopeptides and /or their salts may be
36 used in the composition. In this instance it is preferred
37 that those containing the sequence A-B-C-D-E above
38 predominate.

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1 The phosphopeptide or mixture of phosphopeptides is
2 preferably substantially pure at least to the extent of not
3 containing unpalatable impurities.

4 The following phosphopeptides have been found to be
5 useful in the compositions of the present invention:-

6 T1.Glu-Met-Glu-Ala-Glu-Pse-Ile-Pse-Pse-Pse-Glu-Glu-Ile-Val-
7 Pro-Asn-Pse-Val-Glu-Gln-Lys,

8 T2.Glu-Leu-Glu-Glu-Leu-Asn-Val-Pro-Gly-Glu-Ile-Val-Glu-Pse-
9 Leu-Pse-Pse-Pse-Glu-Glu-Ser-Ile-Thr-Arg,

10 T3.Asn-Thr-Met-Glu-His-Val-Pse-Pse-Pse-Glu-Glu-Ser-Ile-Ile-
11 Pse-Gln-Glu-Thr-Tyr-Lys,

12 T4.Asn-Ala-Asn-Glu-Glu-Glu-Tyr-Ser-Ile-Gly-Pse-Pse-Pse-Glu-
13 Glu-Pse-Ala-Glu-Val-Ala-Thr-Glu-Glu-Val-Lys, and

14 T5.Glu-Gln-Leu-Pse-Pth-Pse-Glu-Glu-Asn-Ser-Lys.

15 The amino acid symbols are as follows : Pse-
16 phosphoserine, Ser-Serine, Pth-phosphothreonine, Thr-
17 threonine, Glu-glutamate, Asp-aspartate, Ala-alanine, Asn-
18 asparagine, Gln-glutamine, Gly-glycine, Arg-arginine, His-
19 histidine, Ile-isoleucine, Leu-leucine, Lys-lysine, Met-
20 methionine, Pro-proline, Tyr-tyrosine, Val-valine.

21 The phosphopeptide may be made synthetically by
22 chemical synthesis or genetic engineering or can be
23 extracted from naturally occurring material.

24 Because of cost considerations it is currently more
25 economic to extract the phosphopeptide from casein and in
26 particular from alpha-s casein or beta-casein. Phosvitin
27 may also be used as a source of the peptide. Further,
28 phosphoproteins in cereals, nuts and vegetables particularly
29 in bran husks or sheaths may be used to produce the peptide
30 above. In particular, rice, wheat, oat, barley or rye
31 brans. Soybean and meat contain phosphoproteins which may
32 be of use in obtaining the peptide above.

33 Casein and in particular alpha-s casein or beta-casein
34 or salts thereof such as sodium caseinate contain
35 polypeptides which can be cleaved to simpler peptides.
36 Such cleavage may be effected by digestion, such digestion
37 may be chemical or proteolytic.

38 It is presently preferred to digest casein with one of

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1 trypsin, pepsin, chymotrypsin, papain, thermolysin or
2 pronase. Of these, trypsin is preferred.

3 The digested casein can be fractioned into peptides
4 including the sequence A-B-C-D-E and other peptides. The
5 presence of said other peptides is not deleterious to
6 efficacy, however, certain of said other peptides have
7 objectionable taste and accordingly if any of said other
8 peptides are to be included it is preferable to remove those
9 having objectionable taste. In general, those of said
10 other peptides having objectionable taste seem to be
11 hydrophobic.

12 The following peptides have been found to have
13 objectionable taste:-

- 14 1. Glu-Val-Leu-Asn
- 15 2. Asn-Glu-Asn-Leu-Leu
- 16 3. Ala-Pro-Phe-Pro-Gln-Val-Phe-Gly
- 17 4. Leu-Arg-Phe
- 18 5. Phe-Phe-Val-Ala-Pro-Phe-Pro-Gln-Val-Phe-Gly-Lys
- 19 6. Leu-Arg-Leu
- 20 7. Phe-Tyr-Pro-Glu-Leu-Phe

21 (Glu-glutamate; Val-valine; Leu-leucine; Asn-asparagine;
22 Ala-alanine; Pro-proline; Phe-phenylalanine; Gln-glutamine;
23 Gly-glycine; Arg-Arginine; Lys-lysine; Tyr-tyrosine.)

24 Preferably the peptide is one exhibiting a reduction in
25 hydroxy apatite dissolution rate of at least 15% under the
26 test conditions defined herein.

27 Preferably the peptide is one exhibiting a reduction
28 in hydroxy apatite dissolution rate of at least 25% under
29 the test conditions defined herein.

30 Preferably, the peptide is one exhibiting a reduction
31 in hydroxy apatite dissolution rate of at least 30% under
32 the test conditions defined herein.

33 Preferably, the peptide is one exhibiting a reduction
34 in hydroxy apatite dissolution rate of at least 32% under
35 the test conditions defined herein.

36 Preferably, the peptide is present as 0.01 to 10% by
37 weight.

38 Preferably, the peptide is present as 0.01 to 5% by

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1 weight.

2 Preferably, the peptide is present as 0.01 to 2% by
3 weight.

4 The composition of this invention may be in the form of
5 a comestible such as foodstuff or confectionery, dentifrice,
6 tablet or comprise a pharmacologically acceptable vehicle or
7 solution of suspension for topical application to the teeth
8 or gingival tissues or a mouthwash. Other modes of
9 administering the peptide would be acceptable if
10 physiologically or pharmacologically acceptable.

11 Of particular interest as compositions are chewing gum,
12 breakfast foods, ice-cream and other frozen confectionery,
13 confectionery, sweets and cakes as these are all known as
14 caries problem materials. Similar considerations apply to
15 other potentially cariogenic food components.

16 Also of particular interest are dentifrices,
17 mouthwashes and preparations for topical application to
18 teeth and gingival tissue and enteric capsules for the
19 treatment of bone disorders and mineral malabsorption.

20 Also of interest is the use of compositions in
21 accordance with this invention in respect of dental
22 treatment of cavities. In this last respect, there appears
23 to be evidence of remineralization of incipient lesions
24 which are considered to be a pre-cavity condition. However,
25 there is also evidence to indicate that application of
26 compositions in accordance with this invention to the
27 surfaces of actual cavities and to surfaces of teeth
28 produced by removal of decay material from actual cavities
29 or by fracture is beneficial.

30 Since a topical application of a composition in
31 accordance with this invention which is an aqueous solution
32 to surfaces of actual cavities or surfaces of teeth produced
33 by removal of decay material from actual cavities or by
34 fracture is unlikely to have long term effect, we have
35 further sought to provide compositions which might have the
36 desired long term effect.

37 Accordingly, the present invention also provides a
38 composition in accordance with this invention and adapted to

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1 remain in contact with a tooth surface over a prolonged
2 period. The invention also provides methods and means for
3 maintaining compositions in accordance with this invention
4 in contact with a tooth surface over a prolonged period.

5 In this last respect a prolonged period should be
6 interpreted in accordance with the effect desired and the
7 time taken to achieve sufficient of that effect to be of
8 value. However, in some instances that prolonged period may
9 be as short as one day but is more preferably a period of
10 weeks or months.

11 In one instance a tooth cavity is coated with a
12 composition in accordance with this invention and the cavity
13 is closed to restrict escape of the composition. Such
14 closure may be effected by capping or use of dental cavity
15 filling compositions.

16 In another instance the composition is so formulated as
17 to be adapted to remain in place for a prolonged period. In
18 this instance the composition of the invention may form part
19 of a dental filling composition.

20 Accordingly, the present invention also provides a
21 dental filling composition comprising a phosphopeptide of
22 formula A-B-C-D-E as defined above and a carrier therefor
23 adapted to adhere the composition to a tooth surface.

24 Such a dental filling composition may contain dental
25 filling materials known per se including amalgams and
26 settable polymers.

27 Of particular interest are dental filling compositions
28 which contain calcium. The calcium is desirably in the form
29 of calcium phosphate or hydroxyapatite.

30 The phosphopeptides for use in the invention can be
31 extracted in a number of ways but the use of a fractionation
32 technique is generally preferred.

33 The phosphopeptides can be extracted by fractionation
34 based on molecular size or charge characteristics. Due to
35 the unique negative charge density and divalent metal ion
36 sequestering ability of the peptides conferred by the active
37 sequence A-B-C-D-E as defined, the preferred fractionation
38 procedure is anion exchange chromatography or selective

1 precipitation or a combination of both.

2 The following procedure illustrates one mode of
3 extraction.

4 Extraction Procedure I.

5 An example of the phosphopeptides are those produced by
6 a tryptic digestion of bovine milk casein. The digestion
7 of whole sodium caseinate or fractions (alpha-S or beta)
8 produced by selective precipitation (Zittle, C.A. and Custer
9 J.H.; J. Dairy Sci 46L 1183-1189, 1983) is carried out using
10 a protein: trypsin ratio of 50:1 in 20 mM Tris HCl pH 8.0,
11 2.5mM NaCl at 37°C for 1h. The peptides were fractionated
12 using a Pharmacia FPLC system with a Mono Q HR 5/5 column
13 and eluted with a NaCl gradient; Buffer A 20mM Tris HCl pH
14 8.0, 2.5mM NaCl; Buffer B 20 mM Tris HCl pH 8.0, 500mM
15 NaCl, gradient 0-100% Buffer B/30 min; flow rate 1ml/min.
16 Fractions were washed and concentrated using an Amicon
17 Ultrafiltration Cell with a UM05 filter. The peptides were
18 identified using a Water Associates PICO-TAG amino acid
19 analysis system using phenylisothiocyanate amino acid
20 derivatisation. Phosphate was measured by the method of
21 Itaya and Ui (Clin. Chim. Acta. 14:381-388, 1960). The
22 peptides were sequenced (after the removal of phosphate by
23 alkaline phosphatase) using manual Edman degradation and the
24 resulting PTH-amino acids identified using reverse phase
25 HPLC on a Zorbax ODS column 25x0.48 cm (DuPont).

26 The following phosphopeptides were individually
27 obtained from a tryptic digestion of sodium caseinate using
28 the above procedure.

29 T1.Glu-Met-Glu-Ala-Glu-Pse-Ile-Pse-Pse-Pse-Glu-Glu-Ile-Val-
30 Pro-Asn-Pse-Val-Glu-Gln-Lys.

31 T2.Glu-Leu-Glu-Glu-Leu-Asn-Val-Pro-Gly-Glu-Ile-Val-Glu-Pse-
32 Leu-Pse-Pse-Pse-Glu-Glu-Ser-Ile-Thr-Arg.

33 T3.Asn-Thr-Met-Glu-His-Val-Pse-Pse-Pse-Glu-Glu-Ser-Ile-Ile-
34 Pse-Gln-Glu-Thr-Tyr-Lys.

35 T4.Asn-Ala-Asn-Glu-Glu-Glu-Tyr-Ser-Ile-Gly-Pse-Pse-Pse-Glu-
36 Glu-Pse-Ala-Glu-Val-Ala-Thr-Glu-Glu-Val-Lys.

37 T5.Glu-Gln-Leu-Pse-Pth-Pse-Glu-Glu-Asn-Ser-Lys.

38 In addition the following peptides were also obtained:

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- 1 T6.Asp-Ile-Gly-Pse-Glu-Pse-Thr-Glu-Asp-Gln-Ala-Met-Glu-Asp-
2 Ile-Lys.
3 T7.Val-Pro-Gln-Leu-Gln-Ile-Val-Pro-Asn-Pse-Ala-Glu-Glu-Arg.
4 T8.Thr-Val-Asp-Met-Glu-Pse-Thr-Glu-Val-Phe-Thr-Lys.
5 T9.Leu-Pth-Glu-Glu-Lys.

6 The peptides T1, T6 and T7 were also obtained from a
7 TPCK-tryptic digest of α_{s1} -caseinate (comprising α_{s1}
8 and α_{s0}). Peptide T2 was also obtained from a TPCK-
9 tryptic digest of beta-caseinate. Peptides T3, T4, T5, T8
10 and T9 were also obtained from a TPCK-tryptic digest of
11 α_{s2} -caseinate (comprising α_{s2} , α_{s3} , α_{s4} and
12 α_{s8}). The amino acid symbols are as follows: Pse-
13 phosphoserine, Ser- serine, Pth-phosphothreonine, Thr-
14 threonine, Glu- Glutamate, Asp- aspartate, Ala- alanine,
15 Asn- asparagine, Gln- glutamine, Gly- glycine, Arg- arginine,
16 His- histidine, Ile- isoleucine, Leu- leucine, Lys- lysine,
17 Met - methionine, Pro- proline, Tyr- tyrosine, Val- valine.

18 Extraction Procedure II

19 The following procedure illustrates one mode of
20 selective precipitation.

21 A solution of sodium caseinate was digested with
22 trypsin (50:1, casein:trypsin) for one hour at 37°C with the
23 pH maintained at 8.0 by the addition of NaOH. HCl (0.1M) was
24 then added to the solution at room temperature to pH 4.7 and
25 the resulting precipitate removed. BaCl₂ was added to the
26 supernatant to a level of 0.25% w/v followed by an equal
27 volume of absolute ethanol and the resulting precipitate was
28 removed and dried. The precipitate was dissolved in one
29 tenth the original volume of water (to facilitate
30 dissolution the pH was raised with NaOH) and the solution
31 acidified to pH 3.5 with 1M HCl. An equal volume of acetone
32 was added and the precipitate removed and dried. The
33 precipitate was then redissolved in H₂O and acidified to pH
34 2.0 by addition of HCl. The resulting precipitate was
35 removed and discarded and the supernatant was adjusted back
36 to pH 3.5 with NaOH and an equal volume of acetone was
37 added. The resulting precipitate was collected, redissolved
38 in water and H₂SO₄ added to precipitate BaSO₄ which was

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1 discarded. The supernatant was then dialysed and
2 lyophilised or spray dried. A mixture of 5 phosphopeptides
3 were obtained with this procedure.

4 The following are the phosphopeptides obtained:-

5 T1.Glu-Met-Glu-Ala-Glu-Pse-Ile-Pse-Pse-Pse-Glu-Glu-Ile-Val-
6 Pro-Asn-Pse-Val-Glu-Gln-Lys.

7 T2.Glu-Leu-Glu-Glu-Leu-Asn-Val-Pro-Gly-Glu-Ile-Val-Glu-Pse-
8 Leu-Pse-Pse-Pse-Glu-Glu-Ser-Ile-Thr-Arg.

9 T3.Asn-Thr-Met-Glu-His-Val-Pse-Pse-Pse-Glu-Glu-Ser-Ile-Ile-
10 Pse-Gln-Glu-Thr-Tyr-Lys.

11 T4.Asn-Ala-Asn-Glu-Glu-Glu-Tyr-Ser-Ile-Gly-Pse-Pse-Pse-Glu-
12 Glu-Pse-Ala-Glu-Val-Ala-Thr-Glu-Glu-Val-Lys.

13 T5.Glu-Gln-Leu-Pse-Pth-Pse-Glu-Asn-Ser-Lys.

14 The ratio of the phosphopeptides (T1:T2:T3:T4:T5) in
15 the final preparation depends on the starting material and
16 conditions of hydrolysis. Digesting sodium caseinate with
17 TPCK-trypsin yields largely T2 with small amounts of T1, T3
18 and T4. However, T2 shows greater lability than the other
19 peptides such that more rigorous digestion as occurs with
20 some commercial casein digests yields a preparation
21 containing largely T1 with small amounts of T3 and T4.

22 If in lieu of sodium caseinate, alpha s1-casein is used
23 for this procedure pure T1 is obtained. With beta-casein as
24 the starting material pure T2 is obtained.

25 The most common sequences of the active peptides is the
26 pentapeptide Pse-Pse-Pse-Glu-Glu. The spacings of the
27 phosphate and carboxyl groups in a beta-conformation of this
28 pentapeptide are shown in Fig 1.

29 The 6.88 Angstrom spacings of phosphates and carboxyls
30 allows specific attachment to calcium atoms along the c-axis
31 of hydroxyapatite crystals. This pentapeptide sequence
32 occurs in peptides T1 to T4 and occurs modified in peptide
33 T5 - Pse-Pth-Pse-Glu-Glu following a conservative
34 substitution of phosphothreonine for phosphoserine.

35 Conservative substitutions within the active sequence
36 would be phosphothreonine and to a lesser extent
37 phosphotryrosine or phosphohistidine for phosphoserine
38 although phosphoserine is preferable. Another possible

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1 substitution for phosphoserine would be glutamate or
2 aspartate but again phosphoserine is preferable. A possible
3 substitution for glutamate is aspartate.

4 The active peptides can sequester calcium phosphate and
5 other salts of divalent metal ions. One mole of T1 binds 18
6 mole of CaHPO_4 such that a 10mg/ml solution of T1 at pH 7.0
7 can solubilize 80mM CaHPO_4 producing a metastable
8 supersaturated solution with respect to calcium phosphate
9 species. With chloride as the counter ion one mole of T1
10 binds only 5 mole of Ca^{++} binding only to serine phosphates.
11 One mole of T1 with about 18 mole of CaHPO_4 bound (M.W.
12 4883) will henceforth be referred to as calcium phosphate
13 T1. An important chemical feature of calcium phosphate T1
14 is that above 2% w/v in water the composition is a
15 thixotropic gel. T1-T5 have been shown to be potentially
16 anticarcinogenic using the following test procedures:

17 Test 1. Hydroxyapatite Dissolution Rate Assay.

18 This test is a modification of a test procedure already
19 described (Reynolds, E.C., Riley, P.F. and Storey, E.
20 Calcif. Tiss Int 34:s52-s58, 1982). The purpose of this
21 test is to determine the effect of the peptides on
22 hydroxyapatite dissolution and in this respect since tooth
23 enamel is largely composed of hydroxyapatite it is believed
24 that useful comparisons can be made.

25 Double distilled, deionized water (18 mega ohms/cm) was
26 used throughout. Analytical reagent grade chemicals were
27 obtained from Ajax Chemicals, Australia. Hydroxyapatite-
28 spheroidal was purchased from BOH. A chromatography column
29 containing 0.1g of hydroxyapatite beads was used for the
30 demineralisation assay. Tris 5mM, pH 8.3 containing 50mM
31 NaCl was used as the column buffer at 20°C and was pumped
32 through the column at a rate of 0.1ml/min. A peptide
33 solution 0.1mg/ml of buffer was applied to the column and
34 0.2ml fractions were collected before and after peptide
35 application and assayed for total calcium, phosphate and
36 peptide. From these values a rate of dissolution (nmol
37 calcium or phosphate per min) for each 0.2ml fraction was
38 obtained.

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1 Phosphopeptides T1-T5 all decreased hydroxyapatite
2 dissolution rate by about 32%.

3 Phosphopeptides T6-T9 were found to be much less
4 effective.

5 Fluoride plus phosphopeptide T1 gave a combined
6 reduction in hydroxyapatite dissolution (40% reduction).
7 The phosphopeptide T1 caused a 50% greater retention of
8 fluoride in the hydroxyapatite column.

9 This work shows that these phosphopeptides bind to
10 hydroxyapatite and reduce the minerals dissolution rate and
11 enhance the retention of fluoride in the crystal matrix.
12 The reduction in hydroxyapatite dissolution was related to
13 the phosphoserine content and spacings within the peptides.

14 Test 2. Intra-Oral Caries Test.

15 The anticariogenicity of phosphopeptide T1 was
16 determined using a modification of the intra-oral caries
17 test of Koulourides and Ostrom (Caries Res. 10:442-482,
18 1978). Enamel slabs were inset in a removable intra-oral
19 appliance to simulate an approximal area. This was done on
20 both sides of the removable appliance (left and right).
21 The appliance was worn to allow plaque accumulation in the
22 simulated approximal areas. Eight times a day the
23 appliance was removed and placed in a solution at 37°C.
24 The solution was 2% w/v sucrose, 2% w/v glucose, 140 mM KCl,
25 20 mM NaCl at pH 7.0. Twice a day the right side enamel
26 slabs received exposure to a solution containing 1.8% w/v
27 calcium phosphate T1 in 140 mM KCl, 20 mM NaCl at pH 7.0,
28 while the left side received only the salt solution. At
29 the completion of the experiment the enamel slabs were
30 removed, sectioned and subjected to microradiography and
31 microhardness testing. The microradiography showed that
32 the slabs exposed to the sugar-salt solution (left-side) had
33 sub-surface, caries-like lesions. However, the slabs
34 exposed to the sugar-salt solution and the peptide T1
35 solution twice a day showed no caries-like changes. The
36 results were confirmed by microhardness analysis. Plaque
37 was also taken from both sides of the appliance and analysed
38 for calcium phosphate, serine phosphate and peptide T1 using

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1 a competitive, quantitative, enzyme-linked immunosorbent
2 assay (ELISA) utilising monoclonal antipeptide T1
3 antibodies.

4 This showed that the plaque on the right side of the
5 appliance exposed twice a day to the peptide T1 solution
6 contained the peptide at a level of at least 0.4% w/wet wt
7 of plaque and the level of calcium phosphate had increased
8 2-4 fold.

9 This work shows that peptide T1 is incorporated into
10 plaque thereby increasing the plaque level of calcium and
11 phosphate so inhibiting the caries process. This method of
12 incorporation and accumulation in dental plaque can be used
13 to carry remineralising and antibacterial ions into plaque
14 and enamel e.g. Ca, PO_4 , FPO_3 , Zn, Cu, Sn, Ag, Al, Fe and
15 La.

16 Test 3 - Intra-Oral Remineralisation

17 An intra-oral appliance similar to that used in the
18 previous test procedure was used except that the enamel
19 slabs had been previously exposed to a demineralising
20 solution to produce two sub-surface demineralised lesions in
21 each slab. The demineralising solution was a 0.1M lactate
22 buffer pH 5.0 containing 500 mg/L hydroxyapatite and 1%
23 agar. The appliances were worn by subjects for 10 days.
24 Twice each day the appliances were removed and a drop of
25 remineralising solution was placed on the enamel slabs on
26 the right of the appliance. The left-side enamel slabs
27 served as controls. After 10 days the enamel slabs were
28 removed, sectioned and subjected to microradiography. The
29 amount of mineral deposited back into the sub-surface
30 lesions was determined using microdensitometry. The
31 remineralising solution containing 1.8% w/v calcium
32 phosphate T1 pH 7.0 returned 57% of the mineral lost
33 compared with 13% by saliva alone.

34 Test 4 - Plaque pH Fall

35 Subjects refrained from oral hygiene for 3-5 days then
36 rinsed with a 5% sucrose solution for 1 min. Plaque samples
37 were removed and pH was measured using the one drop
38 technique. After approximately 5 min the pH fell to around

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1 5.0. However, if the subjects rinsed with a solution
2 containing 1.8% w/v calcium phosphate T1, pH 7.0 15 min
3 before rinsing with the 5% sucrose solution the plaque pH
4 did not fall below 6.7, demonstrating significant pH
5 buffering by the calcium phosphate T1.

6 While the precise mechanism by which the
7 phosphopeptides exhibit anticariogenic activity is not
8 known, the following speculative theories have been put
9 forward but are not to be taken as binding or limiting.

10 The phosphopeptides may accumulate in plaque and
11 enamel, buffer plaque acid, prevent enamel demineralisation
12 and enhance remineralisation. The small molecular weight
13 of the phosphopeptides may allow penetration and
14 accumulation in plaque and enamel pores. The
15 phosphopeptides, due to the appropriate spacing of serine
16 phosphate residues, may bind to tooth enamel mineral and
17 prevent demineralisation. The peptides may also carry
18 calcium and phosphate (fluorophosphate on modification) into
19 plaque and enamel, in an appropriate form, possibly allowing
20 spontaneous remineralisation. The phosphoserine residues
21 may also buffer plaque acid. The phosphopeptide may also
22 carry antibacterial metal ions e.g. Zn, Cu, Sn, Ag, Al, Fe
23 and La into plaque and in this way have an antiplaque and
24 antigingivitis effect. The metal ions are carried by the
25 phosphopeptides primarily due to the phosphoserine residues.
26 Phosphopeptides may bind to plaque bacteria and inhibit
27 sugar utilisation.

28 The ability of these peptides to sequester calcium
29 phosphate can be utilised in the treatment of various
30 rarefying bone diseases. These peptides can significantly
31 increase the absorption of calcium, phosphate and iron in
32 the gut. Hence, pharmaceutical vehicles (e.g. enteric
33 capsules) or foods containing calcium phosphate T1 and
34 ferrous phosphate T1 can be used for the treatment of
35 osteoporosis/osteomalacia and anaemia.

36 Applicants have formulated various compositions in
37 accordance with this invention as follows. In general, the
38 compositions contain from 0.01-10% by weight of

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1 phosphopeptide.

2 Example 1. Flour: In a device for mixing dry
3 substances, 1% by weight of calcium phosphate T1 was blended
4 with flour.

5 Example 2. Cereal: A breakfast cereal was sprayed with
6 a solution of calcium phosphate T1 in water. The cereal
7 flakes were then dried to produce a finished product
8 containing 1% calcium phosphate T1.

9 Example 3. Bread: 1% by weight of calcium
10 phosphate T1 was added to the flour during the mixing of
11 ingredients for the manufacture of bread.

12 Example 4. Cake mix: 1% by weight of calcium
13 phosphate T1 was added to the dry ingredients in the
14 preparation of a cake mix.

15 Example 5. Confectionery: In the preparation of
16 confectionery 1% by weight of calcium phosphate T1 was added
17 to the final mixture.

18 Example 6. Biscuit: In the preparation of a
19 biscuit/mixture 1% by weight of calcium phosphate T1 was
20 added to the other dry ingredients during mixing.

21 Example 7. Beverage: A beverage was prepared in which
22 0.1% weight of calcium phosphate T1 had been dissolved.

23 Example 8. Tablet: A tablet was made containing 10% by
24 weight of calcium phosphate T1 together with excipients
25 being flavouring matter and binding material.

26 In preparation of a typical dentifrice within the scope
27 of this invention, the requisite salt and salts of the
28 selected phosphopeptide are incorporated into dentifrice
29 compositions in any suitable manner depending on whether a
30 powder, paste or liquid preparation is to be produced. For
31 this purpose appropriate preparations of surface-active
32 agents, binders, flavouring materials and other excipients
33 required to achieve the required form of dentifrice are
34 added.

35 The invention is further illustrated by the following
36 examples:

37 Example 9. Tooth paste: A toothpaste was prepared
38 having the following composition:

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1	Calcium phosphate T1	5.0% by weight
2	CMC 4MF	1.0% " "
3	Saccharin 450	0.2% " "
4	Glycerin (B.P.)	25.0% " "
5	Sodium lauryl sulphate	
6	(Empicol 0919)	5.0% " "
7	Sodium benzoate	0.5% " "
8	Flavour 9/893090	0.8% " "
9	Calcium phosphate	1.0% " "
10	Water Deionised	39.5% " "
11	Thixosyl 33J	9.5% " "
12	Syloid AL-1	12.0% " "
13	Titanium Dioxide 3328	0.5% " "

14 Example 10. Toothpaste: A preparation as set out in
 15 Example 9 was repeated but with the addition of 0.2% sodium
 16 fluoride in a suitable form.

17 Example 11. Toothpaste: A preparation as set out in
 18 Example 9 was repeated but with the addition of 0.4%
 19 stannous fluoride in a suitable form.

20 Example 12. Toothpaste: A preparation as set out in
 21 Example 9 was repeated but with the addition of 0.78%
 22 monosodium fluorophosphate in a suitable form.

23 Example 13. Toothpowder: The following preparation was
 24 made:

25	Calcium phosphate T1	5.0% by weight
26	Soluble saccharin	0.1% " "
27	Colour agent	Trace
28	Dibasic calcium phosphate	94.1% " "

29 Example 14. Toothpowder: A preparation as set out in
 30 Example 13 was made but with the addition of 0.78%
 31 monosodium fluorophosphate in a suitable form.

32 Example 15. Liquid dentifrice: A preparation was made
 33 consisting of:

34	Sodium alginate	1.4% by weight
35	Calcium phosphate T1	2.0% " "
36	Sodium lauryl sulphate	1.0% " "
37	Flavouring	Trace
38	Colouring	Trace

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1 Water 95.5% " "

2 Example 16. Liquid dentifrice: As for Example 15 but

3 with 0.5% sodium fluoride added.

4 Example 17. Mouthwash: The following preparation was

5 made:

6	Calcium phosphate T1	2.0% by weight
7	Sodium fluoride	0.5% " "
8	Flavouring	Trace
9	Colouring	Trace
10	Water	97.5% " "

11 Example 18. Carbonated beverage: 0.1% by weight of

12 calcium phosphopeptide T1 was added to a commercially

13 available carbonated beverage.

14 Example 19. Fruit juice: 0.1% by weight of calcium

15 phosphopeptide T1 was added to a commercially available

16 fruit juice.

17 Example 20. Solution for topical application to teeth.

18	Calcium Phosphate T1	2%
19	NaF	0.6 mM
20	ZnAcetate	0.1 mM
21	SrCl ₂	0.1 mM

22 (this solution may be formed into gel by increasing the

23 amount of calcium phosphate T1).

24 Example 21. Dental filling material

25	Calcium phosphate T1	5% w/w
26	Calcium phosphate	95% w/w
27	Polymeriser	trace

28 Made as a paste with water

29 The polymeriser used in this example was

30 glutaraldehyde.

31 Example 22. Dental filling material.

32	Calcium phosphate T1	5% w/w
33	Calcium phosphate	70%
34	Acrylic polymer	25%
35	Catalyst for polymer	trace

36 Example 23. Topical Gel for the treatment of hypersensitive

37 teeth.

38	Calcium phosphate T1	4.0% by weight
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1	SrF ₂	1.0% by weight
2	Flavouring	Trace
3	Water	95%

4 In the above calcium phosphate T1 was used for illustration
5 but in lieu any appropriate phosphopeptide and/or salt might
6 be used.

7 Modifications and adaptations may be made to the above
8 described without departing from the spirit and scope of
9 this invention which includes every novel feature and
10 combination of features disclosed herein.

11 The claims form part of the disclosure of this

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1 THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

2 1. A phosphopeptide or a salt thereof the phosphopeptide
3 having from 5 to 30 amino acids including the sequence

4 A-B-C-D-E

5 where A,B,C,D and E are independently phosphoserine,
6 phosphothreonine, phosphotyrosine, phosphohistidine,
7 glutamate and aspartate.

8 2. A phosphopeptide as claimed in claim 1, wherein A,B and
9 C are independently phosphoserine, phosphothreonine,
10 phosphotyrosine and phosphohistidine and D and E are
11 independently phosphoserine, phosphothreonine, glutamate and
12 aspartate.

13 3. A phosphopeptide as claimed in claim 1, where A,B and C
14 are phosphoserine and D and E are glutamate.

15 4. A phosphopeptide being one of Glu-Met-Glu-Ala-Glu-Pse-
16 Ile-Pse-Pse-Pse-Glu-Glu-Ile-Val-Pro-Asn-Pse-Val-Glu-Gln-Lys.

17 5. A phosphopeptide being one of Glu-Leu-Glu-Glu-Leu-Asn-
18 Val-Pro-Gly-Glu-Ile-Val-Glu-Pse-Leu-Pse-Pse-Pse-Glu-Glu-Ser-
19 Ile-Thr-Arg.

20 6. A phosphopeptide being one of Asn-Thr-Met-Glu-His-Val-
21 Pse-Pse-Pse-Glu-Glu-Ser-Ile-Ile-Pse-Gln-Glu-Thr-Tyr-Lys.

22 7. A phosphopeptide being one of Asn-Ala-Asn-Glu-Glu-Glu-
23 Tyr-Ser-Ile-Gly-Pse-Pse-Pse-Glu-Glu-Pse-Ala-Glu-Val-Ala-Thr-
24 Glu-Glu-Val-Lys.

25 8. A phosphopeptide bein one of Glu-Gln-Leu-Pse-Pth-Pse-
26 Glu-Glu-Asn-Ser-Lys.

27 9. A phosphopeptide or a salt thereof as claimed in any
28 preceding claim and in substantially pure form.

29 10. A mixture of phosphopeptides or salts thereof wherein a
30 phosphopeptide or salt thereof in accordance with any one of
31 claims 1-9 predominates.

32 11. A composition comprising a phosphopeptide or a salt
33 thereof in accordance with any one of claims 1-9 together
34 with a physiologically acceptable diluent.

35 12. A composition as claimed in claim 11, wherein the
36 phosphopeptide or salt thereof is present in the composition
37 as 0.01 to 10% by weight.

38 13. A composition as claimed in claim 11, wherein the

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1 phosphopeptide or salt thereof is present in the composition
2 as 0.01 to 5% by weight.

3 14. A composition as claimed in claim 11, wherein the
4 phosphopeptide or salt thereof is present in the composition
5 as 0.01 to 2% by weight.

6 15. A composition as claimed in any one of claims 11-14,
7 wherein the diluent is a pharmaceutically acceptable
8 diluent.

9 16. A composition as claimed in any one of claims 11-14,
10 wherein the diluent is an orally ingestible material.

11 17. A composition as claimed in claim 16, wherein the
12 diluent is a comestible.

13 18. A composition as claimed in claim 17, in the form of a
14 foodstuff or confection.

15 19. A composition as claimed in any one of claims 11-14, in
16 the form of a toothpaste, tooth powder, dentifrice,
17 mouthwash or preparation for topical application to teeth or
18 gingival tissue.

19 20. A composition as claimed in any one of claims 11-14, in
20 the form of a gel.

21 21. A composition as claimed in any one of claims 11-14, in
22 the form of a dental filling composition.

23 22. A composition as claimed in claim 21 and additionally
24 containing calcium phosphate or hydroxyapatite.

25 23. A method of obtaining a phosphopeptide in accordance
26 with any one of claims 1-9 which comprises fractionating a
27 digest of casein, alpha-s-casein, beta-casein or a salt
28 thereof.

29 24. A phosphopeptide in accordance with anyone of claims 1-
30 9 in combination with calcium phosphate or hydroxy apatite.

31 25. A combination in accordance with claim 24, comprising
32 about 16 mole of CaHPO_4 per mole of phosphopeptide.

33 26. A combination in accordance with claim 24, or claim 25
34 in the form of a solution or gel.

35 27. A phosphopeptide or salt thereof, composition
36 containing same or a method of obtaining same substantially
37 as hereinbefore described with reference to any one of the
38 Examples.

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1 28 The articles, things, parts, elements, steps, features,
2 methods, processes, compounds and compositions referred to
3 or indicated in the specification and/or claims of the
4 application individually or collectively, and any and all
5 combinations of any two or more of such.

Fig. 1

